

Application No.: 10/040430

Docket No.: SUPP-P05-008

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A method for identifying an immunosuppressive agent, comprising:
 - (i) providing a cell containing an NF-ATc polypeptide which is encoded by a nucleic acid that hybridizes under conditions of 5X SSC at 42 °C to SEQ ID NO:45 and has one or more of the following biological activities:
 - (a) binds calcineurin;
 - (b) undergoes nuclear localization upon T cell activation; and
 - (c) activates gene transcription upon T cell activation;
 - (ii) contacting the cell of (i) with a compound that induces nuclear translocation of the NF-ATc polypeptide;
 - (iii) contacting the cell before, during or after step (ii), with a test agent; and
 - (iv) assaying for nuclear translocation of the NF-ATc polypeptide, wherein an inhibition of nuclear transport in the cell relative to a cell that was not contacted with the test agent indicates that the test agent is a candidate immunosuppressive agent.
2. (Previously Presented) The method of claim 1, wherein the assaying for nuclear translocation comprises determining the nuclear presence of the NF-ATc polypeptide.
3. (Previously Presented) The method of claim 1, wherein the assaying for nuclear translocation comprises determining the nuclear association between the NF-ATc polypeptide and an NF-ATn polypeptide.
4. (Previously Presented) The method of claim 1, wherein the assaying for nuclear translocation comprises determining the binding of the NF-AT polypeptide or an NF-ATc:NF-ATn polypeptide complex to an NF-AT DNA binding sequence.
5. (Previously Presented) The method of claim 4, comprising using a gel mobility shift assay to determine the binding of the NF-AT polypeptide or an NF-ATc:NF-ATn polypeptide complex to an NF-AT DNA binding sequence.
6. (Previously Presented) The method of claim 1, further comprising determining the level of expression of a test nucleic acid linked to an NF-AT DNA binding sequence.
7. (Previously Presented) The method of claim 1, wherein the compound of step (ii) stimulates Ca⁺⁺ release in the cell.

Application No.: 10/040430

Docket No.: SUPP-P05-008

8. (Previously Presented) The method of claim 7, wherein the compound is ionomycin.
9. (Previously Presented) The method of claim 1 wherein the cell further comprises an NF-ATn polypeptide and wherein assaying for nuclear translocation includes determining the level of NF-AT complex comprising NF-ATc and NF-ATn, wherein the presence of a lower level of NF-AT complex relative to a cell that has not been contacted with a test agent indicates that the test agent is a candidate immunosuppressive agent.
10. (Previously Presented) The method of claim 9 wherein assaying for nuclear translocation includes determining the level of NF-AT complex bound to an NF-AT binding sequence, wherein the presence of a lower level of bound NF-AT complex relative to that in a cell that has not been contacted with the test agent indicates that the test agent is a candidate immunosuppressive agent.
11. (Previously Presented) A method for identifying an immunosuppressive agent, comprising
 - (i) contacting a purified NF-ATc polypeptide or cell extract containing an NF-ATc polypeptide with: a purified NF-ATn polypeptide, or a cell extract containing an NF-ATn polypeptide, and a test agent, under conditions which permit the formation of an NF-AT complex comprising NF-ATc and NF-ATn, wherein the NF-ATc polypeptide is encoded by
by a nucleic acid that hybridizes under conditions of 5X SSC at 42 °C to SEQ ID NO:45 and has one or more of the following biological activities:
 - (a) binds calcineurin;
 - (b) undergoes nuclear localization upon T cell activation; and
 - (c) activates gene transcription upon T cell activation; and
 - (ii) determining the level of NF-AT complex formed, wherein a lower level of NF-AT complex relative to the level of NF-AT complex formation in the absence of the test agent indicates that the test agent is a candidate immunosuppressive agent.
12. (Previously Presented) The method of claim 11, wherein the NF-ATc or NF-ATn polypeptide is immobilized.
13. (Currently Amended) The method of claim 1 wherein the cell further includes an NF-AT regulated enhancer region linked to a test nucleic acid; and assaying for nuclear translocation includes determining the level of expression of the test ~~gene~~ nucleic

Application No.: 10/040430

Docket No.: SUPP-P05-008

- acid, wherein a lower level of expression of the test gene nucleic acid relative to its level of expression in a cell that was not contacted with the test agent indicates that the test agent is a candidate immunosuppressive agent.
14. (Previously Presented) The method of claim 13, wherein the test gene encodes a protein which is essential for cell proliferation or viability.
15. (Currently Amended) The method of claim 1 wherein the cell further includes an NF-AT regulated enhancer region linked to a test nucleic acid; and assaying for nuclear translocation includes determining the level of expression of the test gene nucleic acid, wherein a higher level of expression of the test gene relative to its level of expression in a cell that was not contacted with the test agent indicates that the test agent is a candidate immunostimulatory agent.
16. (Currently Amended) The method of claim 15, wherein the test gene nucleic acid encodes a protein which is essential for cell proliferation or induces cell death.
17. (Previously Presented) A method for identifying an immune regulating agent, comprising
- (i) contacting a cell or a cell extract containing an NF-ATc polypeptide with a test agent, wherein the NF-ATc polypeptide is encoded by a nucleic acid that hybridizes under conditions of 5X SSC at 42 °C to SEQ ID NO:45 and has one or more of the following biological activities:
 - (a) binds calcineurin;
 - (b) undergoes nuclear localization upon T cell activation; and
 - (c) activates gene transcription upon T cell activation; and
 - (ii) determining the level of phosphorylation of the NF-ATc polypeptide, wherein a difference in the level of phosphorylation relative to that of a cell or cell extract that was not contacted with the test agent indicates that the test agent is a candidate immune regulating agent.
18. (Previously Presented) The method of claim 17, further comprising contacting the cell with an agent which induces the nuclear translocation of the NF-ATc polypeptide.
19. (Previously Presented) A method of any one of claims 1, 9, 10, 13, 15, and 17, wherein the NF-ATc polypeptide is encoded by a heterologous nucleic acid in the cell.
20. (Previously Presented) A method of claim 19, wherein the NF-ATc polypeptide or portion thereof comprises at least 25 amino acids having an amino acid sequence which is substantially identical to an amino acid sequence set forth in SEQ ID NO:46.

Application No.: 10/040430

Docket No.: SUPP-P05-008

21. (Previously Presented) A method of claim 19, wherein the NF-ATc polypeptide or portion thereof is encoded by a nucleic acid which hybridizes to a nucleic acid having the nucleotide sequence set forth in SEQ ID NO:45 or the complement thereof.